Effects of alkyl groups attached to the carbamoyl nitrogen atom of an *N*-arylcarbamoylpyrazoline on insecticidal activity

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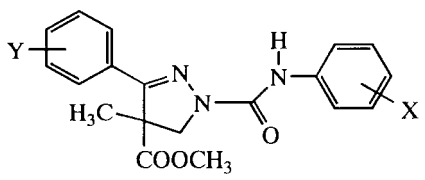
Abstract: *N*-Alkylated analogs (C_1–C_3) of an *N*-arylcarbamoylpyrazoline were prepared. The introduction of these alkyl groups completely changed the crystal structure in respect of the torsion angle of the amide C–N bond of the non-alkyl compound. The introduction of methyl and ethyl groups slightly, and that of an isopropyl group markedly, decreased insecticidal activity against American cockroaches and house flies. Conformational analyses of the compounds suggested that the insecticidally active conformer of this class of compounds is in the *anti*-position regarding the N′–C(=O) and N-aryl bonds in which the non-alkyl compound is energetically the most stable. The most stable conformers of the alkylated compounds are in the *syn*-position, and these compounds would interact with target sites in the less stable *anti*-form.

Keywords: *N*-arylcarbamoylpyrazoline; insecticide; American cockroach; house fly; metabolic dealkylation; conformational effect

1 INTRODUCTION

The *N*-arylcarbamoylpyrazolines[1] and related compounds[2] have potent insecticidal activity against a broad spectrum of insects. After the introduction of various substituents into the pyrazoline ring,[3] compounds of general structure **A** (Fig 1) were found to have good insecticidal properties; these were particularly marked where X was a substituent with electron-withdrawing properties at the *para* position.[4,5] This may suggest that the hydrogen atom of the carbamoyl moiety has certain roles in the interaction within the molecule or with the target site by hydrogen bonding. In addition to these substituent effects, we found that the *S*-isomer regarding the 4-position of the pyrazoline ring is insecticidally more potent than the *R*-isomer.[6,7] These findings encouraged us to prepare compounds where alkyl groups are introduced onto the nitrogen atom of the carbamoyl moiety. A structure–activity analysis of these compounds may reveal the role of the N–H hydrogen atom of the carbamoyl moiety in the insecticidal effect. The introduction of an alkyl group onto the nitrogen atom may additionally affect the conformation of the molecule, resulting in a discontinuous change in the insecticidal potency.



A

Figure 1. General structure of compounds discussed.

In this report, we describe the preparation of some *N*-alkylated compounds at the carbamoyl moiety of one of the representative compounds having structure **A** and their insecticidal activity, which was measured under some synergistic conditions. We discuss possible roles of these alkyl groups in affecting insecticidal activity, based on their metabolic degradability as well as on the conformational change of the compounds.

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2 MATERIALS AND METHODS

2.1 Compounds

The test compounds are listed in Table 1. They were used as racemates for the insecticidal tests. Compound **1**, methyl 3-(4-chlorophenyl)-1-[*N*-(4-chlorophenyl)carbamoyl] - 4 - methyl - 2 - pyrazoline - 4-carboxylate, has been reported previously.[5] Compounds **2**–**4** were derived from compound **1** as described below. Yields were not optimized. Compound structures were confirmed by nuclear magnetic resonance (NMR) spectroscopy measured with a JEOL PMX 60 in deuterochloroform using tetramethylsilane as internal standard. Newly prepared compounds were also confirmed by elementary analyses for C, H and N. Melting points were measured with a Yanagimoto micromelting point apparatus and were uncorrected. Crystal structures of the final compounds were analyzed with a Rigaku AFC-5R diffractometer. NIA 16388 (propargyl propyl benzenephosphonate; NIA), which is an inhibitor of the hydrolytic metabolism of pyrethroids,[8,9] was as used in our previous study.[10] Reagent-grade piperonyl butoxide (PB) was used as an inhibitor of oxidative metabolism. NIA and PB were dissolved in methanol and stored in a freezer.

*2.1.1 Synthesis of methyl 3-(4-chlorophenyl)-1-[N-(4-chlorophenyl)-N-methylcarbamoyl]-4-methyl-2-pyrazoline-4-carboxylate (compound **2**)*

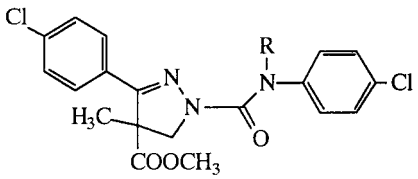
Sodium hydride (0.4 g; 60% in oil, 10 mmol) was treated with hexane twice to remove the oil. The residue was suspended in *N*,*N*-dimethylformamide (DMF, 8 ml), and compound **1** (1.88 g, 4.6 mmol) was added portionwise at room temperature. After hydrogen evolution had ceased, methyl iodide (2.13 g, 15 mmol) in DMF (5 ml) was added dropwise and the mixture was stirred at room temperature. Two hours later, a further suspension prepared as above from sodium hydride (0.2 g, 5 mmol) and DMF (5 ml) was added to the reaction mixture. After stirring at room temperature for 30 min, diethyl ether (50 ml) and water (30 ml) were added to the mixture under ice-cooling. The mixture was separated and the aqueous layer was extracted with ether. The ether layers were combined and washed with water once and with saturated sodium chloride solution three times. The organic layer was dried over magnesium sulfate and concentrated. The residue was purified by column chromatography over silica gel using hexane + ethyl acetate (7 + 3 by volume) as eluent to give compound **2** (0.98 g, 50%), mp 134–135°C. [^{1}H]NMR δ ppm: 1.51 (3H, s, CH_3), 3.38 (3H, s, CH_3-N), 3.69 (3H, s, CH_3-O), 3.89 and 4.43 (2H, d, $J = 11$ Hz, CH_2), 7.0–7.6 (8H, m, aromatic). Calculated for $C_{20}H_{19}Cl_2N_3O_3$: C, 57.16; H, 4.56; N, 10.00 (%); found C, 57.25; H, 4.56; N, 10.07 (%).

*2.1.1 Synthesis of methyl 3-(4-chlorophenyl)-1-[N-(4-chlorophenyl)-N-ethylcarbamoyl]-4-methyl-2-pyrazoline-4-carboxylate (compound **3**) and its N-isopropyl analog (compound **4**)*

Compounds **3** and **4** were similarly prepared from compound **1** by treatment with ethyl iodide and isopropyl iodide, respectively. The yield of compound **3** was 0.34 g (21%) from compound **1** (1.5 g), mp 90°C. [^{1}H]NMR δ ppm: 1.21 (3H, t, $J = 6.9$ Hz, CH_3-CH_2), 1.49 (3H, s, CH_3-C), 3.70 (3H, s, CH_3-O), 3.73–3.91 (2H, m, CH_3-CH_2), 3.91 and 4.37 (2H, d, $J = 11.4$ Hz, CH_2), 7.03–7.33 (8H, m, aromatic). Calculated for $C_{21}H_{21}Cl_2N_3O_3$: C, 58.08; H, 4.87; N, 9.67 (%); found C, 58.08; H, 4.82; N, 9.75 (%).

The yield of compound **4** was 0.21 g (9.4%) from compound **1** (2 g), mp 103–104°C. [^{1}H]NMR δ ppm: 1.18 (6H, dd, $(CH_3)_2$-CH), 1.45 (3H, s, CH_3-C), 3.69 (3H, s, CH_3-O), 3.88–4.35 (2H, d, $J = 11.1$ Hz, CH_2), 4.77 (1H, sept, $J = 6.9$ Hz, $(CH_3)_2$-CH, 7.02–7.34 (8H, m, aromatic). Calculated

Table 1. Insecticidal activities of *N*-alkyl pyrazolines against American cockroaches and house flies



Compound No.	R	American cockroaches log(1/MLD) (mol)				House flies log(1/LD50) (mol)	
		Alone	+ PB	+ NIA	+ (PB + NIA)	Alone	+ (PB + NIA)
1	H	8.83	8.93	9.03	9.08	9.25	9.56
2	CH_3	8.47	8.62	8.52	8.32	8.45	8.31
3	C_2H_5	8.59	8.70	8.70	8.30	8.75	8.24
4	i-C_3H_7	7.87	7.97	7.67	7.67	8.23	7.65

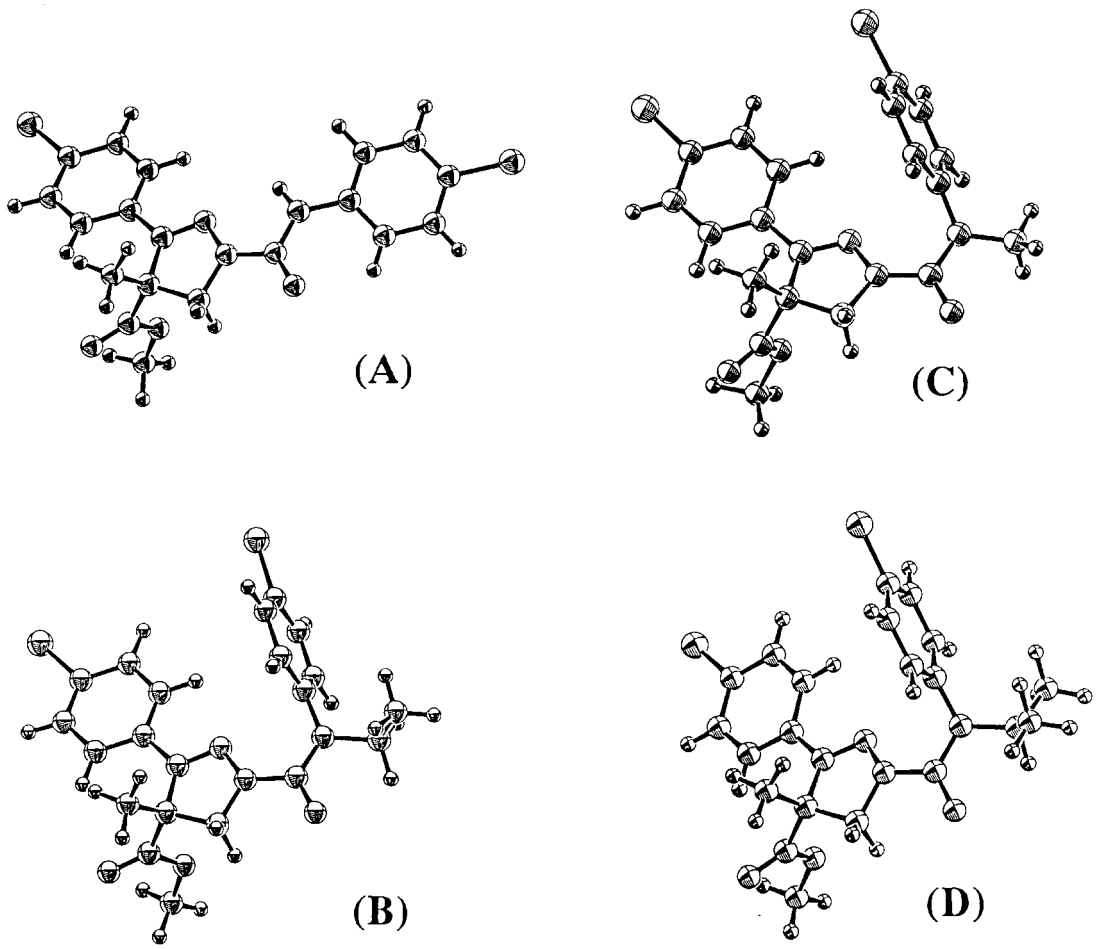


Figure 2. Crystal structures of *S*-isomers of compounds **1** (A), **2** (B), **3** (C) and **4** (D).

for $C_{22}H_{23}Cl_2N_3O_3$: C, 58.94; H, 5.17; N, 9.37 (%); found C, 59.04; H, 5.16; N, 9.49 (%).

2.2 Insecticidal tests

2.2.1 American cockroaches

The insecticidal activity of test compounds was measured against male adult American cockroaches, *Periplaneta americana* L, one to three months old, by the procedure described previously.[5,10] Various volumes (1–10 µl) of a methanol solution of each compound were injected into the abdomen of the insects so as to make the doses at 0.1 intervals in log units. In some experiments, a methanol solution (1 µl) containing PB (50 µg) or NIA (50 µg) or both was injected into the abdomen as synergist(s) 1 h before the injection of test compounds to suppress the metabolic mechanism. Three insects were used to test each dose of each compound. Injected insects were kept at 20(±1)°C for 24 h. The minimum dose at which two of the three insects died or were paralyzed was considered the minimum lethal dose (MLD; mole). Methanol (1–10 µl) with or without synergists did not affect the MLD value. For each series of experiments, at least 25 insects were used. The log(1/MLD) values for the test compounds are listed in Table 1. The standard error of the value was ±0.1.

2.2.2 House flies

Adult females, three to seven days old, of susceptible house flies (*Musca domestica* L, CSMA) were anesthetized by carbon dioxide gas. A methanol solution (1 µl) containing various amounts of the compounds was topically applied to the ventral side of the abdomen. To suppress the metabolic mechanism, 1 µl of a methanol solution containing PB (2 µg) plus NIA (2 µg) was applied to the abdomen immediately before the topical application of the insecticides. Methanol alone in this volume and synergists alone in these amounts had no toxic effect on the flies. The lethal effect, defined as the dropping of the insects due to convulsion and/or paralysis to the bottom of the container, was monitored at intervals at 25(±1)°C. Twenty insects were used for each dose of each compound. Regardless of whether synergistic or non-synergistic conditions were used, the increase in the number of affected insects ceased about 8–10 h after the application of the insecticides. The dose-response relationship was therefore examined from data at 15 h after the application. From such a relationship, the LD_{50}, ie the dose (mole) at which 50% of the insects were killed, was calculated by probit transformation. The $\log(1/LD_{50})$ values are given in Table 1. The standard error of the potency was within ±0.2 for three runs.

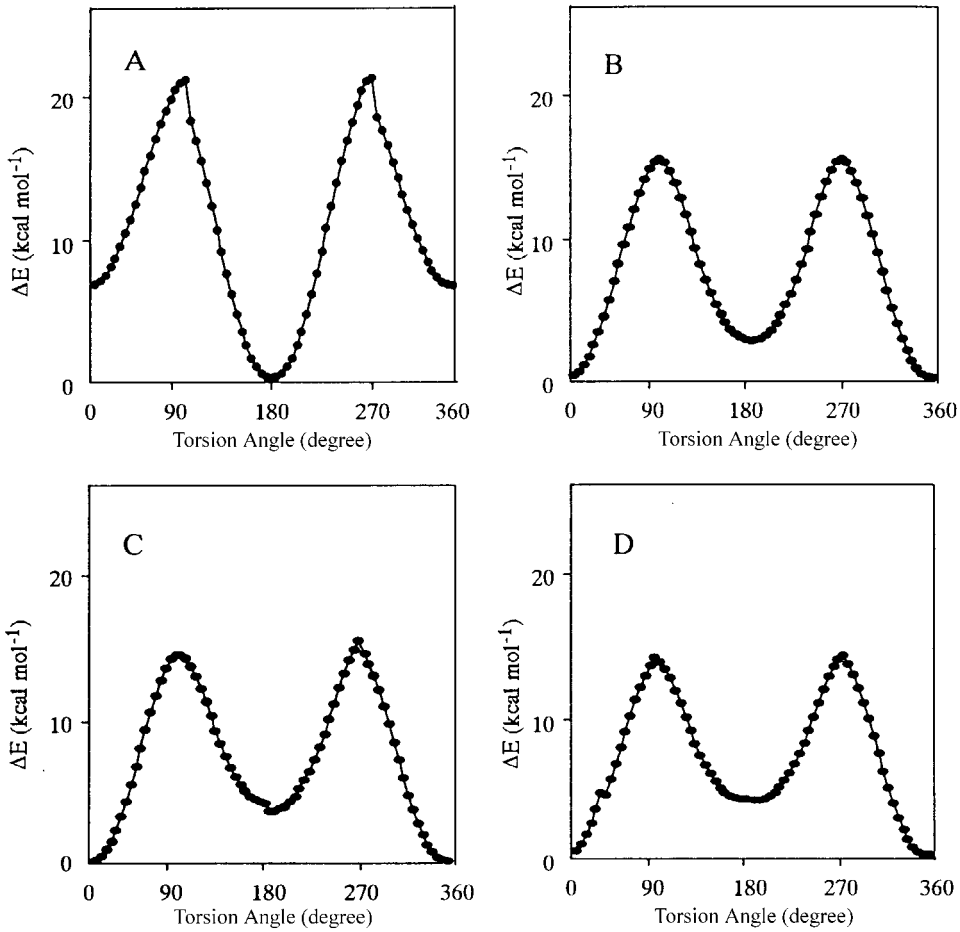


Figure 3. Conformation-energy plots of compounds **1** (A), **2** (B), **3** (C) and **4** (D). Torsion angles are defined in the text. ΔE shows the energy for each conformer of each compound relative to that having the minimum energy among the conformers.

2.3 Molecular modelling

All computations were done with a QUANTA96 graphics program with CHARMm Ver. 23.2 molecular force field (Molecular Simulations, Inc, CA, USA). Distance-dependent dielectric constants were used to assess electrostatic interactions. The crystal structures of compounds **1**–**4** were fully optimized first with 200 steps of the steepest-descent method, followed by minimization with up to 2000 steps with the adaptive-based Newton–Rapson (ABNR) algorithm. The C(=O)–NR bond of the carbamoyl moiety where R is H or an alkyl group was first rotated so as to give the *anti* conformation regarding the N′–C(=O) and NH-aryl bonds, the torsion angle of which was defined as 180°, and was systematically rotated from 0° to 360° in 5° increments. Each structure was energetically minimized by an ABNR minimizer.

3 RESULTS

3.1 Effects of substituents and synergists on insecticidal activities

Compound **1** was the most potent among the tested compounds against the American cockroach, under both synergistic and non-synergistic conditions (Table 1). Without the synergists, compounds **2** and **3** were slightly less potent than compound **1**, and compound **4** was the least active. A mixture of PB and NIA increased the activity of compound **1**, but slightly decreased that of compounds **2**–**4**. Under synergistic conditions with a mixture of PB and NIA, compound **4** was the least active among the tested compounds and was less active than compound **1** by 1.4 in log units. Compounds **2** and **3** were less active than compound **1** by about 0.8 in log units.

The activity values determined against house flies showed a trend similar to that against American cockroaches (Table 1). The mixture of PB and NIA increased the activity of compound **1** but not of others.

3.2 X-Ray crystal structures of pyrazolines

Racemates were used for the X-ray analyses. The crystal structures of the *S*-isomer of each compound are shown in Fig 2. The *S*-isomer of compound **1** has a roughly planar structure regarding the two benzene rings and the pyrazoline ring (Fig 2A). The C(=O)–NH bond of the amide moiety takes an angle so that the positions of these two benzene rings are apart. The two benzene rings in each of compounds **2**–**4**, however, come close and are roughly perpendicular to each other (Fig 2B–D).

3.3 Conformational analysis of pyrazolines

The profiles of relative conformational energies as a function of rotation around the C(=O)–NR bond, where R is H or an alkyl group, for compounds **1–4** are given in Fig 3. The most stable conformer of compound **1** is in the *anti*-position, whereas the other three *N*-alkyl compounds (**2–4**) have a local minimum at their *syn*-position. These observations are consistent with the results of the X-ray crystallographic analyses.

4 DISCUSSION

Effects of the synergists on the insecticidal activity against the American cockroach were not at a similar level among the tested compounds (Table 1). Since we used PB and NIA to suppress oxidative and hydrolytic mechanisms of insects, respectively, we could estimate the respective metabolic activity as a difference, Δ[log(1/MLD)], of the insecticidal values measured with synergist(s) from that without synergists.[11] The Δ[log(1/MLD)] values shown in Fig 4 are small, but may be useful to draw some conclusions about the metabolic activity of the cockroach.

The enhancing effect of NIA on the insecticidal activity of compound **1** was greater than that of PB (Fig 4). Among the synergistic conditions used, the simultaneous injection of these two synergists was the most effective for this compound. For compounds **2** and **3**, the synergistic effects of NIA alone or a mixture of PB and NIA on the activity were somewhat different from those for compound **1**. For these two compounds, the increment of log (1/MLD) by the use of NIA was the same as or slightly smaller than those measured with PB. A mixture of these two synergists clearly lowered the insecticidal activity comparing with that measured without synergists or with other synergistic conditions. Even with NIA alone, the activity of compound **4** was lower than that without synergists or with PB.

The positive deflection of the Δ[log(1/MLD)] value in Fig 4 by use of synergist(s) indicates that the metabolic mechanism(s) was inhibited by synergist(s) to give a higher activity than that measured without synergists. Contrarily, the negative deflection as seen with compounds **2–4** probably indicates that the metabolic mechanisms of insects to transform compound **1** were somewhat inhibited by synergist(s). Among the metabolic inhibitors, NIA probably inhibited the cleavage of the C–N bond of the *N*-alkyl moiety of compounds **2–4**, as suggested for imidacloprid and related compounds,[12] besides the inhibition of hydrolysis of the methoxycarbonyl group at position 4 of the pyrazoline ring, as in pyrethroid insecticides.[9,10] Thus, the deflection caused by NIA is most probably due to inhibition of the hydrolysis of the COOMe group of compound **1** or due to the summation of the inhibition of hydrolytic and dealkylating metabolic effects in compounds **2–4**. The isopropyl group of compound **4** might not be removed as easily as other alkyl groups of compounds **2** and **3**, because the activity value of compound **4** only was negatively deflected with NIA. These suppositions can be extended to the insecticidal activity against house flies.

Although the single crystal structures of compounds **2–4** are entirely different from that of compound **1** (Fig 2), the insecticidal activities of compounds **1–3**, particularly against cockroaches, were not very different from each other (Table 1). This may indicate that the conformers obtained from crystallographic analyses are different from those interacting with the target site in aqueous conditions. We have speculated that the conformer with the *anti*-position in respect to the C(=O)–NH bond of compound **1** is the insecticidally active one.[6] A compound with a *para*-chlorophenylacetyl group instead of the *N*-arylcarbamoyl group of compound **1** was, however, more than 100 times less active than compound **1**, presumably because of a high rotational

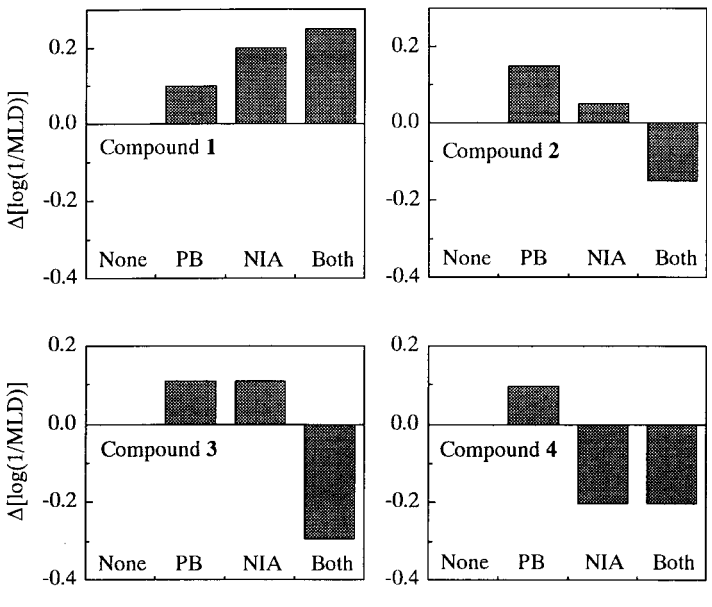


Figure 4. Synergistic effects of PB and NIA on the insecticidal activity of compounds **1–4** against American cockroaches. The Δ[log(1/MLD)] value indicates the deflection of the log(1/MLD) value measured with synergist(s) from that measured without the synergists. Insecticidal data listed in Table 1 were used for the calculation.

ability of the molecule around the C(=O)–CH_2 bond.[6] Although the most stable conformer of compounds **2**–**4** in respect to the C(=O)–N(alkyl) bonds is in the *syn*-position, their *anti*-conformers were the second most stable, and the energy differences of compounds **2**, 3 and **4** between *syn*- and *anti*-conformers were 2.47, 3.56 and 3.97 kcal/mol, respectively (Fig 3). These differences seem to be small enough to allow these compounds to exist in the insecticidally active *anti*-positions. Compound **4** was the least active among the tested compounds (Table 1). This may be due to the lowest probability existing in the *anti*-conformer (Fig 3) or may be due to the unfavorable steric interaction of the bulky isopropyl group with the receptor site.

In summary, the most stable conformer of the nonalkyl compound (**1**) is in the *anti*-position, whereas those of the alkylated compounds (**2**–**4**) are in the *syn*-position. However, the alkylated compounds can also take the *anti*-position at a certain level of probability. In addition, the apparent activities of the alkylated compounds might be due partly to the compound being delakylated by the metabolic system of insects. Thus, the active conformer of the alkylated compounds, even though it was not the most stable in crystals, is thought to be the extended and planar conformer that the non-alkyl compound takes as the most stable conformer.

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